Report

Abandoning Aggression but Maintaining Self-Nonself Discrimination as a First Stage in Ant Supercolony Formation

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Summary

An ant supercolony is a very large entity with very many queens. Although normal colonies of small extent and few queens remain distinct, a supercolony is integrated harmoniously over a large area [1, 2]. The lack of aggression is advantageous: Aggression is costly, involving direct and indirect losses and recognition errors [3, 4]. Indeed, supercolonial ants are among the ecologically most successful organisms [5–7]. But how supercolonies arise remains mysterious [1, 2, 8]. Suggestions include that reduced within-colony relatedness or reduced self-nonself discrimination would foster supercolony formation [1, 2, 5, 7, 9–12]. However, one risks confusing correlation and causality in deducing the evolution from distinct colonies to supercolonies when observing established supercolonies. It might help to follow up observations of another lack of aggression, that between single-queened colonies in some ant species. We show that the single-queened *Lasius austriacus* lacks aggression between colonies and occasionally integrates workers across colonies but maintains high within-colony relatedness and self-nonself discrimination. Provided that the ecological framework permits, reduced aggression might prove adaptive for any ant colony irrespective of within-colony relatedness. Abandoning aggression while maintaining discrimination might be a first stage in supercolony formation. This adds to the emphasis of ecology as central to the evolution of cooperation in general [13].

Results and Discussion

Whereas fierce fights occur between single-queened ant colonies as a rule [1, 14, 15], we compiled scattered literature observations of the lack of aggression between workers of different single-queened colonies for 21 ant species (see the Supplemental Data available online). In ants, cooperation occurs within self, the colony, but the boundary against nonself, other colonies, is well guarded because the transmission of self genes is maximized when the dilution of self is prevented [1]. Hence, across the self-nonself boundary aggression normally occurs.

We dissected one of the hitherto underplayed cases, the underground-living ant *Lasius austriacus*. From queen morphology and colony excavations [16], colonies were inferred to be single queened, and within-colony relatedness was thus inferred to be high. We explored the self-nonself boundary for 16 colonies from four populations in terms of genetic relatedness, signal chemistry, self-nonself discrimination, and interactions within self and across the self-nonself boundary. Distances between colonies were at least 10 m (neighboring colonies) and covered five orders of magnitude (Figure 1).

Data on nine highly variable microsatellites (12.2 ± 4.4 alleles) revealed high relatedness within colonies and low relatedness between colonies, with significant differences between self and nonself over all distances (Figure 1). Genotype inspection showed that the majority of workers within colonies (71 of 80; the remaining nine, from seven colonies, are discussed below) had alleles attributable to a single queen mated once, supporting the suggestion of single-queened colonies [16].

The self-nonself boundary was reflected in gas chromatograms of body surface hydrocarbons, which, in

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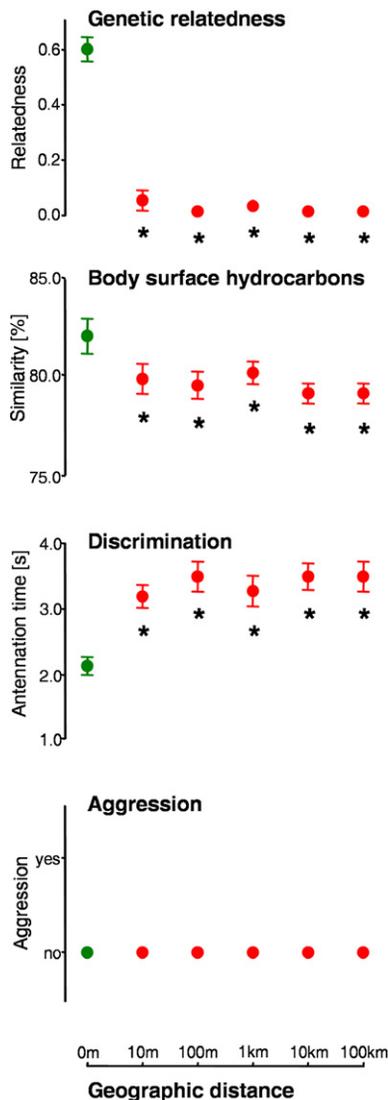[9]These authors contributed equally to this work.

Figure 1. The Self-Nonself Boundary for 16 Colonies of the Ant *Lasius austriacus*

Self is indicated by green, and nonself is indicated by red; means and standard errors are depicted. "*" indicates a significant difference ($\alpha = 0.05$) between self and nonself, as revealed by Bonferroni-Holm adjusted Student's t test with the Satterthwaite approximation. The genetic relatedness panel shows the relatednesses within and between colonies, based on nine microsatellites. The body surface hydrocarbons panel shows Bray-Curtis similarity index values for 26 body surface hydrocarbons measured by gas chromatography. The discrimination panel shows the antennation-bout lengths in one-on-one worker encounters. The aggression panel shows the presence ("yes") or absence ("no") of aggression in one-on-one worker confrontations.

ants, mediate the discrimination of self against nonself [14]. In an analysis of ranked similarities randomization, the grouping of hydrocarbons was stronger according to colony identity (global R_{col} = 0.228, p = 0.001) than to individual identity within colonies (global R_{ind} = 0.080, p = 0.002) or to population identity (global R_{pop} = 0.087, p = 0.004). After the comparison of similarity index values, significant differences between self and nonself emerged at all distances (Figure 1).

We also found that *L. austriacus* is capable of discriminating self against nonself. Video analysis of one-on-one worker encounters revealed significantly shorter antennation bouts in self-self than in self-nonself pairings (Figure 1). In a second experiment, bioassays indicated the existence of chemically cued discrimination because workers lingered significantly more frequently (p < 0.0001) on self than on nonself body surface hydrocarbon extracts when offered the choice with the solvent as control.

In one-on-one worker confrontations, we invariably observed aggression between *L. austriacus* and four other ant species (some active underground, others aboveground), which represented all community dominance levels. Combats were frequently begun by *L. austriacus* (from 51 ± 29% to 66 ± 20% of fights). Encounters between two *L. austriacus* workers, however, never yielded aggression, irrespective of the distance between the home colonies. To test for context dependency, we repeated all of the latter tests in underground conditions, and we again recorded no aggression.

Moreover, we observed integrative behavior across the self-nonself boundary, but this behavior is normally confined to contacts within the colony in which it renews bonds for cooperation. In the laboratory, group formation occurred between any colony fragments put together (eight of eight tests, each with fragments of four colonies), as did food exchange within and between colonies (seven and eight exchanges, respectively, in 40 trials). Integrative behavior across the self-nonself boundary, if not restricted to laboratory conditions, should in nature result in the integration of workers from other colonies. In scrutinizing the field data, we compared microsatellite alleles of the nine workers, mismatching the single-queen pattern to the corresponding alleles in other colonies. Two workers of one colony shared a mother and father with workers from a colony ten meters away. Considering the polymorphism of the nine microsatellite markers, the probability of a haphazard match is p = 4.29×10^{-17}. Because we have sampled only one of the neighboring colonies of each colony, it is likely that the seven other aberrant workers also stemmed from neighboring colonies. We infer that the integration of nonself workers occurs under natural conditions.

Our data show that *Lasius austriacus* perceives self-nonself cues but behaves nonaggressively and even integratively at the self-nonself boundary. Our sampling scheme and the lack of aggression regardless of geographic distance excludes the fact that the nonaggressive behavior is due to reduced aggression between neighbors, compared to strangers. The driving forces for peacefully bridging the boundary could be part of the ecological framework and namely involve the nature of the food source. *Lasius austriacus* is strictly subterranean, tending mealybugs inside nests for honeydew [16]. Because this food can be permanently tapped without the need for long-distance foraging [17], this might reduce both the interaction between colonies and the tension at the self-nonself boundary [4]. The potential benefits of aggression might be low relative to its costs in such cases of reduced between-colony competition [18–20], as was suggested in essence for situations of increased competition [21] in which the same ratio of costs and benefits of aggression might apply, although

the absolute values would be expected to be much higher then.

Social strategies that reduce intragroup relatedness through the adoption of foreigners might appear to be nonadaptive. But the incorporation of additional nonreproductive workers can augment the workforce. Also, the loss of workers to another colony might be outweighed through the avoidance of erroneous self rejection [4, 22]. The lack of aggression against colonies of the same species might save resources, which can be invested in colony growth and reproduction, thus increasing colony fitness, as argued for supercolonies [5]. From the vantage point of the individual, integration into a nonself colony seems even less adaptive because then the worker's genes are dissimilar to those transmitted. However, the reciprocal exchange of workers can render this a stable strategy. This finding adds *L. austriacus* to the wide range of social organisations of *Lasius*, which includes supercoloniality in two species (reviewed by [1, 2]), and confirms the genus as an ideal system for exploring social evolution.

The consistency of nonaggressive behavior despite the recognition of the boundary in *Lasius austriacus* makes it improbable that recognition errors [4] trigger nonaggressive, integrative behavior. The same might apply to the lack of intraspecific aggression in another 20 single-queened, phylogenetically diverse ant species with various ecologies, including activity centers from underground to trees and food sources from scattered to centralized (Supplemental Data). In some of these species, integration as well as aggression occurs. All of this suggests that the relevant benefit-cost ratio rather than a species-specific trait turns the scales to aggressive or integrative behavior at the self-nonself boundary. This awareness could pave the way to an unexpected explanation of supercolonies.

Supercolonies have evolved multiply and via various routes [1, 2, 6–12, 22–24], and finding a general principle for supercolony formation proves difficult [2]. Possibly, lumping the result of the independent pathways under the term supercolonies—ecologically most significant, as exemplified by their infamous invasion success [5, 7, 8, 12]—might give us little chance to disentangle causal and correlated traits.

The social organization of integration despite discrimination in single-queened ants bears the one trait by which supercolonies are defined [1, 2]: worker exchange. Concerning the collateral traits of supercolonies—equally low relatedness within and between nests, lack of discrimination, and lack of aggression—only the latter is shared. This indicates that the lack of aggression in supercolonies might primarily have evolved through neither reduced relatedness nor reduced discrimination.

In principle, reduced relatedness within self as found in multiple-queened ants could be causal in supercolony evolution [1, 2, 9], in that for such nests, the integration of nonself individuals does not lower relatedness and dilute nest identity by much, reducing the initial barrier to worker exchange. However, native and invasive cases in which some species have both single-queened and supercolonial populations (e.g., *Formica*, *Myrmica*, *Solenopsis* [1, 10, 25]) argue against relatedness reduction's being crucial to supercolony evolution. The existence of single-queened sibling species of supercolonial

ants further weakens the view that supercolonial species evolved from multiple-queened ones [1, 24].

Reduced self-nonself discrimination could also initiate the cessation of aggression [2, 5, 7, 8, 12]. Although it was originally proposed for bottleneck situations after anthropogenic introductions [7], it was later suggested that the breakdown of discrimination could also be triggered by natural disturbances [10] or by the evolution of a green-beard mechanism whereby recognition and peaceful acceptance of the bearer of a specific allele irrespective of genetic relatedness is understood [1, 11]. In at least one species, though, individuals within supercolonies can discriminate between nestmates and nonnestmates [9, 22], suggesting that the lack of discrimination might not be initial in their formation.

We argue that the abandonment of aggression itself could be the first stage on the route to harmony. Under some circumstances, this might result in social organizations like that of *L. austriacus*. Under other circumstances, this might be followed by supercolony evolution, resulting in a wide range of native and invasive supercolonies because of the wide range of phylogenetic constraints and ecological settings. Although until now supercolonies were viewed as a byproduct of other processes, we propose that the adaptive value of avoiding the costs of aggression is so strong that under appropriate ecological conditions, it will promote the elimination of territorial aggression. The simplicity of this scenario and the case of *L. austriacus* that illustrates the principle make the argument intuitively plausible, although it is not possible to test our pathway at present because we do not know of a single case of supercolony evolution in progress. It remains open whether the harmony of *Lasius austriacus* has limits and reverts to aggression under some conditions, but the issue is not relevant to our scenario: In both native and invasive ants, both unlimited [26, 27] and limited integration occurs, the latter resulting in aggression between supercolonies of the same species [8, 9]. We recognize that our findings could tend to the modification of the view [7, 8, 28] that the pathways to supercolony necessarily differ between native and invasive species, thus adding to recent, independent evidence along the same lines [6]. Our scenario, however, does not so much challenge or replace previous insights on native and invasive supercolonies as it does complement them by offering a broader view. If our scenario proves generally important, then practical implications also emerge for invasion biology, in that many more species than are currently thought to be potentially invasive might in fact have the potential to become so. Our scenario is also timely in a broader context, considering recent findings from a diverse range of organisms, including wasps, spiders, and bacteria [29–31], on how competition is overcome: The circumstances are very diverse, as are the mediating mechanisms, but ecology might be central to the evolution of cooperation in a competitive world [13].

Experimental Procedures

Sample

Excavations revealed that neighboring colonies of *Lasius austriacus* are about 10 m apart. We collected 300 workers from each of 16 colonies, four each from four populations (with two dyads of

neighboring colonies 100 m apart) in eastern Austria, resulting in intercolonial distances of approximately 10, 100, 1,000, 10,000, and 100,000 m. We also collected 100 workers each from two colonies of four other formicine species, three epigaeic, having various dominance hierarchy positions (*Formica pratensis*, top; *Lasius alienus*, medium; *Plagiolepis vindobonensis*, bottom; [25]), and one hypogaeic (*Lasius flavus*; [25]). We kept all ants in the laboratory for 3 weeks, following [16].

Genetics

Following [32], we extracted the DNA of five workers per *L. austriacus* colony and performed microsatellite analysis for La33b, La33c, La34c, La34e, La35g, La35h, La36a, and La36d. We also used microsatellite primers "La35eF," 5′-CGTTTGCCTCCTTGTTTTG-3′, and "La35eR," 5′-AAGCTTCAAGGACGACAAGG-3′ (GenBank, EF599953). By using KinGroup v2_01212b [33], we calculated the pairwise relatedness for all 3,160 possible pairs of workers, within and across colonies, implementing the estimator of [34]. The allele frequencies were calculated from the colonies sampled. In genotype inspections by eye, we regarded workers as sharing a mother and father when for all nine loci the number of alleles was $\leq$ three and one allele was shared by all workers. Following logic similar to [35], we calculated the probability that two unrelated workers might be sufficiently similar genetically to appear to be full sisters by assuming equal allele frequencies for the nine microsatellites typed, which have 6, 8, 9, 9, 12, 16, 16, 17, and 17 alleles. The probability of two unrelated individuals appearing to be full sisters is $(1/6 \times 2/6) \times (1/8 \times 2/8) \times (1/9 \times 2/9) \times (1/9 \times 2/9) \times (1/12 \times 2/12) \times (1/16 \times 2/16) \times (1/16 \times 2/16) \times (1/17 \times 2/17) \times (1/17 \times 2/17) = 4.29 \times 10^{-17}$.

Behavior

We performed one-on-one worker encounters following [8] but used glass vials of 2 cm diameter, with Fluon-coated walls, combining *L. austriacus* with the above four ant species, with 20 trials each. We observed six behaviors: ignoring, antennation, avoidance, gaster flexion, biting, and fighting; we scored the former three as nonaggressive and the latter three as aggressive. Over the 5 min observation periods, we took down the numbers and inducers of aggressive interactions.

Analogous one-on-one worker encounters were performed with *L. austriacus*, with 32 trials for self (same colony) and 16 trials for each of the five distances of nonself (other conspecific colonies), resulting in 112 encounters. We recorded antennation-bout lengths with video documentation. We repeated the encounters, mimicking underground conditions, in 2 × 1 × 0.5 cm red film-covered cavities of soil from native colonies, testing the soil from both colonies each, totaling 112 encounters. Following [36], in eight tests across all geographic distances between colonies, we combined colony fragments (50 workers) from four colonies marked with honeybee paints for 24 hr and took down any group formation. To analyze food exchange, we kept marked workers for 24 hr, in one treatment without food and in another treatment with honeywater. In eight trials per distance, totaling to 40 trials, we placed one starving, one fed concolonial, and one fed allocolonial worker (different honeywater dyes) together and subsequently detected any food exchange by analyzing the gaster of killed ants.

We performed blind bioassays by using body surface hydrocarbon extracts. We successively extracted five workers in 100 μl hexane, 100 μl ethyl acetate, and 100 μl ethanol for 90 s each and then pooled the extracts. We covered the bottom of 60 glass vials of 2 cm diameter each with 1 μl paraffin oil as a keeper substance. We applied 1 μl solvent to one 120 degree sector of each bottom, as a control, to the second sector 1 μl self extracts, and to the third 1 μl nonself extracts. We performed two trials per vial, involving one worker from each of the two extracted colonies, under dark conditions. Every 15 min, we took down the worker's position (control, self, nonself), during a 10 s period of light, with 16 repeats (1,920 observations). After each observation, we turned the vial 120 degrees and forced the worker to move to another sector.

Chemistry

We extracted five workers per colony separately for 90 s with 25 μl of a mixture of 50% hexane, 45% ethyl acetate, and 5% ethanol including 0.00025% heptacosane. Gas chromatography (GC) was performed on a GC8000 (Fisons Instruments, Italy; flame ionization detector [FID]; DB-5 column diameter 0.25 mm, length 30 m, film 0.25 μm; carrier: hydrogen; 1 μl split-splitless injection; 60°C for 2 min, 15°C min^{-1} to 200°C and 4°C min^{-1} to 320°C for 5 min). Profile synchronization was achieved with retention times. Gas chromatography-mass spectrometry (MS) analyses were run on the GC8000 with quadrupole mass spectrometer, with the above column (carrier: helium; 60°C for 3 min, 15°C min^{-1} to 200°C and 3°C min^{-1} to 320°C for 5 min; electron impact [EI] mode at 70eV; full-scan mode 50–655 amu; scan time: 1.8 s). With MS data, substances were determined as hydrocarbons. MS data were also used so that the GC synchronization could be checked. Peak intensities were calculated by the integration of peak areas with Chrom-Card (Fisons Instruments).

Data Analysis

We followed [37] in calculating the similarity in the relative hydrocarbon intensities between each of the 3,160 possible pairs of workers with the Bray-Curtis similarity index implemented in PRIMERv5 (Plymouth, UK). We applied Student's t test by using the Satterthwaite approximation implemented in SAS9 (SAS Institute [Cary, NC]) and the Bonferroni-Holm correction [38] to compare intracolonial data with data of each of the five geographic distances, for relatedness, antennation-bout length, and Bray-Curtis values of hydrocarbons and to compare the summed values of the 16 observations for the self and the nonself sector in the bioassays. We used the analysis of ranked similarities randomization in PRIMERv5 to test the probability that the pairwise similarities of hydrocarbons within and between populations and colonies, as well as within and between individual identities, were the same. For this we randomly allocated numeric identities, 1–5, to the individuals of each colony.

Supplemental Data

One table is available at http://www.current-biology.com/cgi/content/full/17/21/1903/DC1/.

References

1. Crozier, R.H., and Pamilo, P. (1996). Evolution of Social Insect Colonies (New York: Oxford University Press).
2. Bourke, A.F.G., and Franks, N.R. (1995). Social Evolution in Ants (Princeton, New Jersey: Princeton University Press).
3. Davies, N.B., and Houston, A.I. (1984). Territory economics. In Behavioral Ecology: An Evolutionary Approach, J.R. Krebs and N.B. Davies, eds. (Oxford: Blackwell Scientific), pp. 148–169.
4. Reeve, H.K. (1989). The evolution of conspecific acceptance thresholds. Am. Nat. *133*, 407–435.
5. Holway, D.A., Suarez, A.V., and Case, T.J. (1998). Loss of intraspecific aggression in the success of a widespread invasive social insect. Science *282*, 949–952.
6. Pedersen, J.S., Krieger, M.J.B., Vogel, V., Giraud, T., and Keller, L. (2006). Native supercolonies of unrelated individuals in the invasive Argentine ant. Evolution Int. J. Org. Evolution *60*, 782–791.
7. Tsutsui, N.D., Suarez, A.V., Holway, D.A., and Case, T.J. (2000). Reduced genetic variation and the success of an invasive species. Proc. Natl. Acad. Sci. USA *97*, 5948–5953.
8. Giraud, T., Pedersen, J.S., and Keller, L. (2002). Evolution of supercolonies: The argentine ants of southern Europe. Proc. Natl. Acad. Sci. USA *99*, 6075–6079.

9. Holzer, B., Chapuisat, M., Kremer, N., Finet, C., and Keller, L. (2006). Unicoloniality, recognition and genetic differentiation in a native *Formica* ant. J. Evol. Biol. *19*, 2031–2039.

10. Elias, M., Rosengren, R., and Sundström, L. (2005). Seasonal polydomy and unicoloniality in a polygynous population of the red wood ant *Formica truncorum*. Behav. Ecol. Sociobiol. *57*, 339–349.

11. Krieger, M.J.B. (2005). To b or not to b: A pheromone-binding protein regulates colony social organization in fire ants. Bioessays *27*, 91–99.

12. Errard, C., Delabie, J., Jourdan, H., and Hefetz, A. (2005). Intercontinental chemical variation in the invasive ant *Wasmannia auropunctata* (Roger) (Hymenoptera Formicidae): A key to the invasive success of a tramp species. Naturwissenschaften *92*, 319–323.

13. Foster, K.R., and Xavier, J.B. (2007). Cooperation: Bridging ecology and sociobiology. Curr. Biol. *17*, R319–R321.

14. Vander Meer, R.K., and Morel, L. (1998). Nestmate recognition in ants. In Pheromone Communication in Social Insects: Ants, Wasps, Bees and Termites, M.D. Breed, K.E. Espelie, R.K. Vander Meer, and M.L. Winston, eds. (Boulder, Colorado: Westview Press), pp. 79–103.

15. Pirk, C.W.W., Neumann, P., Moritz, R.F.A., and Pamilo, P. (2001). Intranest relatedness and nestmate recognition in the meadow ant *Formica pratensis* (R.). Behav. Ecol. Sociobiol. *49*, 366–374.

16. Steiner, F.M., Schlick-Steiner, B.C., Schödl, S., Espadaler, X., Seifert, B., Christian, E., and Stauffer, C. (2004). Phylogeny and bionomics of *Lasius austriacus* (Hymenoptera, Formicidae). Insectes Sociaux *51*, 24–29.

17. Pontin, A.J. (1978). The numbers and distribution of subterranean aphids and their exploitation by the ant *Lasius flavus* (Fabr.). Ecol. Entomol. *3*, 203–207.

18. Katzerke, A., Neumann, P., Pirk, C.W.W., Bliss, P., and Moritz, R.F.A. (2006). Seasonal nestmate recognition in the ant *Formica exsecta*. Behav. Ecol. Sociobiol. *61*, 143–150.

19. Mabelis, A.A. (1979). Wood ant wars. Neth. J. Zool. *29*, 451–620.

20. Downs, S.G., and Ratnieks, F.L.W. (2000). Adaptive shifts in honey bee (*Apis mellifera* L.) guarding behavior support predictions of the acceptance threshold model. Behav. Ecol. *11*, 326–333.

21. Ross, K.G., and Keller, L. (1995). Ecology and evolution of social organization: Insights from fire ants and other highly eusocial insects. Annu. Rev. Ecol. Syst. *26*, 631–656.

22. Chapuisat, M., Bernasconi, C., Hoehn, S., and Reuter, M. (2005). Nestmate recognition in the unicolonial ant *Formica paralugubris*. Behav. Ecol. *16*, 15–19.

23. Hölldobler, B., and Wilson, E.O. (1990). The Ants (Cambridge, Massachusetts: Harvard University Press).

24. Wilson, E.O. (1971). The Insect Societies (Cambridge, Massachusetts: Harvard University Press).

25. Seifert, B. (2007). Die Ameisen Mittel- und Nordeuropas (Tauer, Germany: lutra Verlags- und Vertriebsgesellschaft).

26. Schlick-Steiner, B.C., Steiner, F.M., Stauffer, C., and Buschinger, A. (2005). Life history traits of a European *Messor* harvester ant. Insectes Sociaux *52*, 360–365.

27. Tschinkel, W.R. (2006). The Fire Ants (Cambridge, Massachusetts: Harvard University Press).

28. Tsutsui, N.D., and Suarez, A.V. (2003). The colony structure and population biology of invasive ants. Conserv. Biol. *17*, 48–58.

29. Sumner, S., Lucas, E., Barker, J., and Isaac, N. (2007). Radio-tagging technology reveals extreme nest-drifting behavior in a eusocial insect. Curr. Biol. *17*, 140–145.

30. Evans, T.A. (1999). Kin recognition in a social spider. Proc. R. Soc. Lond. B. Biol. Sci. *266*, 287–292.

31. Brockhurst, M.A., Buckling, A., and Gardner, A. (2007). Cooperation peaks at intermediate disturbance. Curr. Biol. *17*, 761–765.

32. Steiner, F.M., Arthofer, W., Schlick-Steiner, B.C., Crozier, R.H., and Stauffer, C. (2007). Eleven microsatellite loci in the sociobiologically enigmatic ant *Lasius austriacus* (Hymenoptera: Formicidae). Mol. Ecol. Notes *7*, 498–500.

33. Konovalov, D.A., Manning, C., and Henshaw, M.T. (2004). KINGROUP: A program for pedigree relationship reconstruction and kin group assignments using genetic markers. Mol. Ecol. Notes *4*, 779–782.

34. Milligan, B.G. (2003). Maximum-likelihood estimation of relatedness. Genetics *163*, 1153–1167.

35. Tay, W.T., and Crozier, R.H. (2001). Mating behaviour of *Rhytidoponera* sp. 12 ants inferred from microsatellite analysis. Mol. Ecol. *10*, 167–173.

36. Astruc, C., Malosse, C., and Errard, C. (2001). Lack of intraspecific aggression in the ant *Tetramorium bicarinatum*: A chemical hypothesis. J. Chem. Ecol. *27*, 1229–1248.

37. Schlick-Steiner, B.C., Steiner, F.M., Höttinger, H., Nikiforov, A., Mistrik, R., Schafellner, C., Baier, P., and Christian, E. (2004). A butterfly's chemical key to various ant forts: Intersection-odour or aggregate-odour multi-host mimicry? Naturwissenschaften *91*, 209–214.

38. Holm, S. (1979). A simple sequentially rejective multiple test procedure. Scand. J. Stat. *6*, 65–70.